

Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

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Analysis of RNA

A number of methods have been developed to quantitate, measure the size of, and map the 5' and 3' termini of specific mRNA molecules in preparations of cellular RNA. These include:

- *Northern hybridization (RNA blotting)*, in which the size and amount of specific mRNA molecules in preparations of total or poly(A)⁺ RNA are determined (Alwine et al. 1977, 1979). The RNA is separated according to size by electrophoresis through a denaturing agarose gel and is then transferred to activated cellulose (Alwine et al. 1977; Seed 1982b), nitrocellulose (Goldberg 1980; Thomas 1980; Seed 1982a), or glass or nylon membranes (Bresser and Gillespie 1983) (see below). The RNA of interest is then located by hybridization with radiolabeled DNA or RNA followed by autoradiography.
- *Dot and slot hybridization*, in which an excess of radiolabeled probe is hybridized to RNA that has been immobilized on a solid support (Kafatos et al. 1979; Thomas 1980; White and Bancroft 1982). Densitometric tracings of the resulting autoradiographs can allow comparative estimates of the amount of the target sequence in various preparations of RNA.
- *Mapping RNA using nuclease S1 or ribonuclease*, in which the precise positions of the 5' and 3' termini of the mRNA and the locations of splice junctions can be rigorously determined (Berk and Sharp 1977; Weaver and Weissmann 1979). Labeled or unlabeled RNA or DNA probes derived from various segments of the genomic DNA are hybridized to mRNA, often under conditions favoring the formation of DNA:RNA hybrids (Casey and Davidson 1977). The products of the hybridization are then digested with nuclease S1 or RNAase under conditions favoring digestion of single-stranded nucleic acids only. Analysis of the digestion products by gel electrophoresis yields important quantitative and qualitative information about the mRNA structure.
- *Primer extension*, in which a small radiolabeled fragment of DNA is hybridized to the mRNA and used as a primer for reverse transcriptase. The resulting product should extend to the extreme 5' terminus of the mRNA, and thus the size of the product reflects the number of nucleotides from the position of the label to the 5' terminus of the mRNA.
- *Solution hybridization*, in which the absolute concentration of the sequence of interest is calculated from the rate of hybridization of a small amount of a specific radioactive probe with a known quantity of purified cellular RNA (see, e.g., Roop et al. 1978; Durnam and Palmiter 1983). Alternatively, an excess of a radiolabeled probe is incubated with a known amount of RNA. The concentration of the RNA of interest can then be estimated from the amount of radioactivity that becomes resistant to nuclease S1 (see, e.g., Favaloro et al. 1980; Beach and Palmiter 1981; Williams et al. 1986).

- *Filter hybridization*, in which purified cellular RNA is end-labeled with ^{32}P and hybridized to a large excess of the homologous DNA that has been immobilized on a solid support (Williams et al. 1986).

Below we describe northern hybridization. Dot and slot hybridization of both crude and purified preparations of RNA are described beginning on page 7.53; nuclease-S1 and RNAase analysis of specific hybrids, beginning on pages 7.58 and 7.71, respectively; and analysis of mRNA by primer extension, beginning on page 7.79.